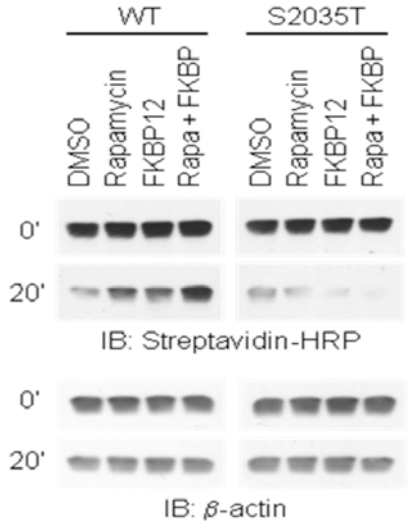
SUPPLEMENTARY INFORMATION

Supplementary Note

SMER3 prevents the assembly of SCF^{Met30} or induces SCF complex dissociation. Presently we are unable to distinguish between these two possible modes of action by SMER3. Attempts to detect SMER3-dependent release of Met30 from the SCF complex *in vitro* were unsuccessful. Furthermore, complex assembly cannot be studied *in vitro* since Met30 expressed without Skp1 is unstable and does not assemble with the purified Cdc53/Skp1 complex. Nevertheless, the fact that SMER3 inhibits SCF^{Met30} ubiquitin E3 ligase activity *in vitro* and causes dissociation of Met30 from Skp1 *in vivo* suggests that SMER3 changes the binding characteristics of Met30 to Skp1, whereby ligase activity is inhibited *in vitro,* but also leads to disassembly *in vivo.*

Destabilization of non-target proteins in DARTS. We found that whereas SMER3 binding protects the F-box region of Met30 (but not Cdc4) from protease digestion, the WD-40 repeat domain of Met30 is rendered more susceptible to protease digestion in the presence of SMER3. We have also observed this destabilization of non-target proteins involving other small molecules. A most striking example is shown below, which involves a well-studied small molecule-protein pair. In this case, rapamycin destabilizes a rapamycin-resistant point mutant of mTOR that cannot bind rapamycin, whereas the wild type mTOR protein is expectedly stabilized by rapamycin.



Discussion about the link between TOR and SCF^{Met30}. Since TOR inhibition does not result in accumulation of deubiquitinated Met4, the genetic interaction between TOR and SCF^{Met30} must be occurring downstream of this event. There are a number of viable hypotheses that can explain our observations. One such hypothesis simply ties the two pathways together based on their convergence on regulation of the G1 cyclins.

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Inhibition of SCF^{Met30} by SMER3 results in activation of Met4 and, consequently, down-regulation of *CLN1* and *CLN2* (ref 14). This is also evident from our gene expression profiling experiments in which expression of the G1 cyclins is seen to be down-regulated upon SMER3 treatment (Supplementary Table 2). Similarly, rapamycin treatment also results in down-regulation of *CLN1* and *CLN2* (ref 24). Another hypothesis, which is not necessarily incongruent with the above, involves a transcription regulatory protein called VDE. VDE has been shown to localize to the nucleus upon TOR inhibition (in a manner similar to that of a well-known TOR-regulated transcription factor, Gln3) [31] and regulate a number of genes involved in sulfur metabolism during sulfur starvation [32, 33]. In fact, one such gene example, *CYS3*, is largely regulated by the Met4 transcription complex, yet VDE binding is shown to enhance gene expression. Furthermore, a number of *MET* genes (that are regulated by Met4) contain the 5' UTR consensus sequence shown to bind VDE. This hypothesis is very interesting (though it remains to be substantiated) since it suggests that while there is a separate response for sulfur starvation from TOR inhibition (including, carbon and/or nitrogen starvation), TOR inhibition can enhance this response through nuclear localization of VDE, resulting in the co-regulation of genes (i.e. genes that result in down-regulation of the G1 cyclins) during times of nutrient depletion.

Yeast strains and media. Yeast strains used in this study are listed below. Cells were grown in standard YPD (yeast extract-peptone-dextrose), YPDA (YPD+adenine), or SD (synthetic dextrose) dropout media lacking the appropriate amino acid as indicated. Standard protocols for yeast genetics and molecular biology were used [34].

Strain	Relevant Genotype	Source or Reference
BY4741	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	[35, 36]
met4Δ	As BY4741 but *met4Δ::kanMX4*	this study
PY1	MATa *bar1Δ ura3Δns ade1 his2 leu2-3,112 trp1-1a*	[37]
cdc4-3 (PY187)	As PY1 but *cdc4-3*	[38]
cdc34-3 (PY24)	As PY1 but *cdc34-3*	[38]
cdc53-1 (PY175)	As PY1 but *cdc53-1*	[38]
met4Δ (PY544)	As PY1 but *met4::KAN*	[39]
met30-6 (PY283)	As PY1 but *met30-6::KAN*	[39]
met30-9 (PY281)	As PY1 but *met30-9::KAN*	[38]
skp1-25 (PY1374)	As PY1 but *skp1-25-YCp::TRP1 skp1::KAN*	this study
13Myc-MET30 SKP1-HA (PY1277)	As PY1 but ^{13Myc}*MET30::ZEO SKP1*^{HA}*::KAN pep4::URA3*	this study
HBTH-SKP1 (PY1822)	As PY1 but ^{HBTH}*SKP1 lys2::ZEO arg4::HYG pep4::KAN*	this study
GAL1-SKP1 (PY204)	As PY1 but *SKP1*-YIpG2*::LEU2 skp1::KAN*	this study
GAL1-MET30-RGS6H (PY162)	As PY1 but *MET30*^{RGS6H}-YIpG2*::LEU2::TRP1 met30::KAN*	this study

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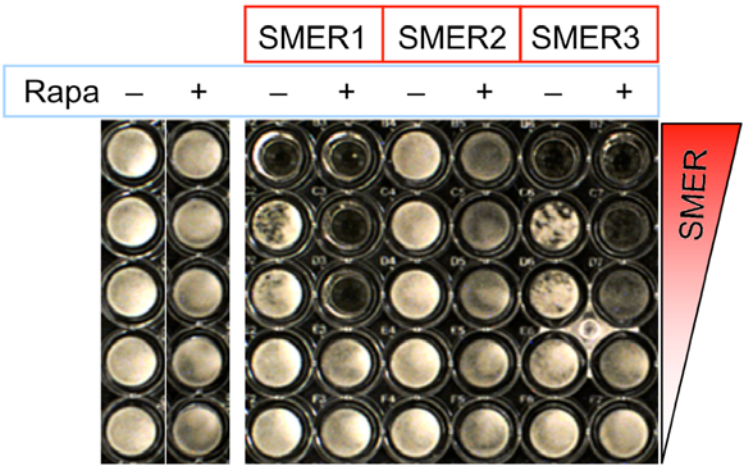
Supplementary Figures



Supplementary Figure 1. Synthetic sick/lethal interactions between SMER3 and rapamycin, where cells treated with both molecules display more severe fitness defects than cells treated with either molecule alone. BY4741 cells (4 x 10^5 cells/ml) were treated with SMERs serially diluted 2-fold from 50 μM to 3.125 μM in the presence and absence of 50 nM rapamycin. The plate was imaged 2 days post-treatment.

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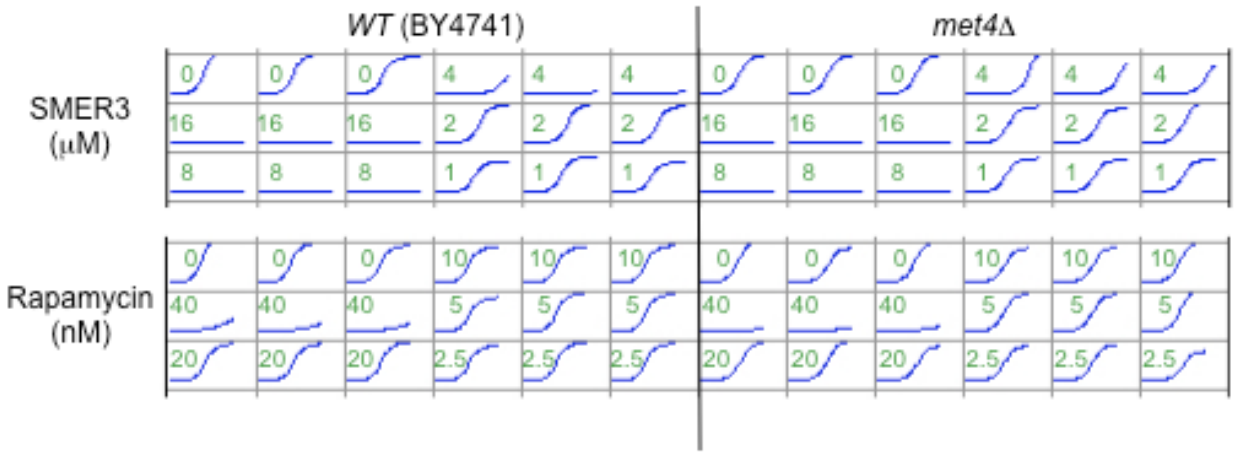

Supplementary Figure 2. Growth curve analysis of SMER3 resistance in *met4*Δ cells. The numbers in green indicate SMER3 or rapamycin concentrations in each well. Three independent colonies were used in this experiment.

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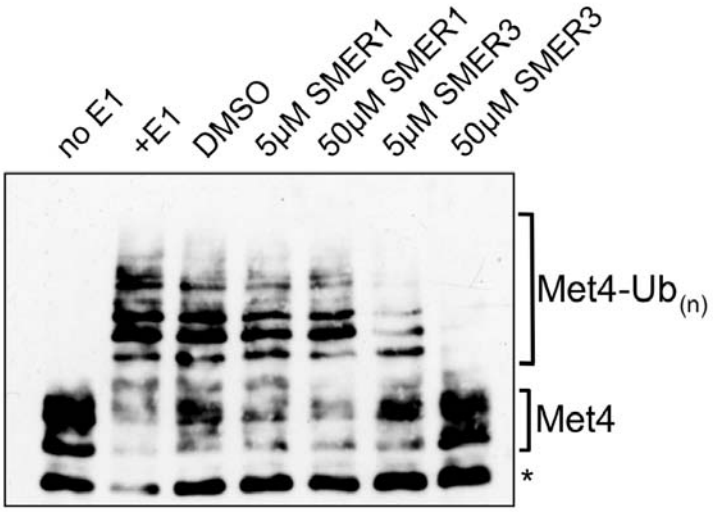

Supplementary Figure 3. SMER3, but not SMER1, inhibits SCF^{Met30} E3 ligase *in vitro*. Components of SCF^{Met30} were co-expressed in insect cells and the complex was purified based on a GST-tag fused to Skp1. Met4 expressed in insect cells was bound to SCF^{Met30} and the ligase-substrate complex was eluted with glutathione. Purified ligase-substrate complexes were pre-incubated with DMSO or the indicated concentrations of SMER1 or SMER3 for 15 min before the ubiquitination reaction was initiated by addition of a final concentration of 250 nM yeast E1 (Boston Biochem, #E-301), 4 μM recombinant Cdc34 purified from *E. coli* [46], 5 mM ATP, and 80 μM ubiquitin (Sigma, #U6253). The reaction was allowed to proceed for 60 minutes and reaction products were analyzed by immunoblotting with anti-Met4 antibody. The asterisk (*) indicates Met30, which cross-reacts with the anti-Met4 antibody.

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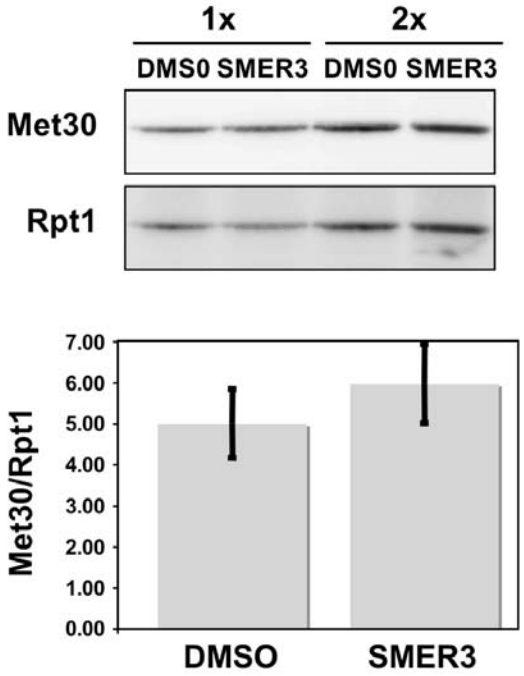

Supplementary Figure 4. Total Met30 protein levels were unaffected after treatment of cells with 20 μM SMER3 for 30min. Quantitation was done on a Fuji LAS4000 imaging system. Samples were normalized to the proteasome subunit Rpt1. 1x and 2x indicates total amounts of lysates loaded on the gel.

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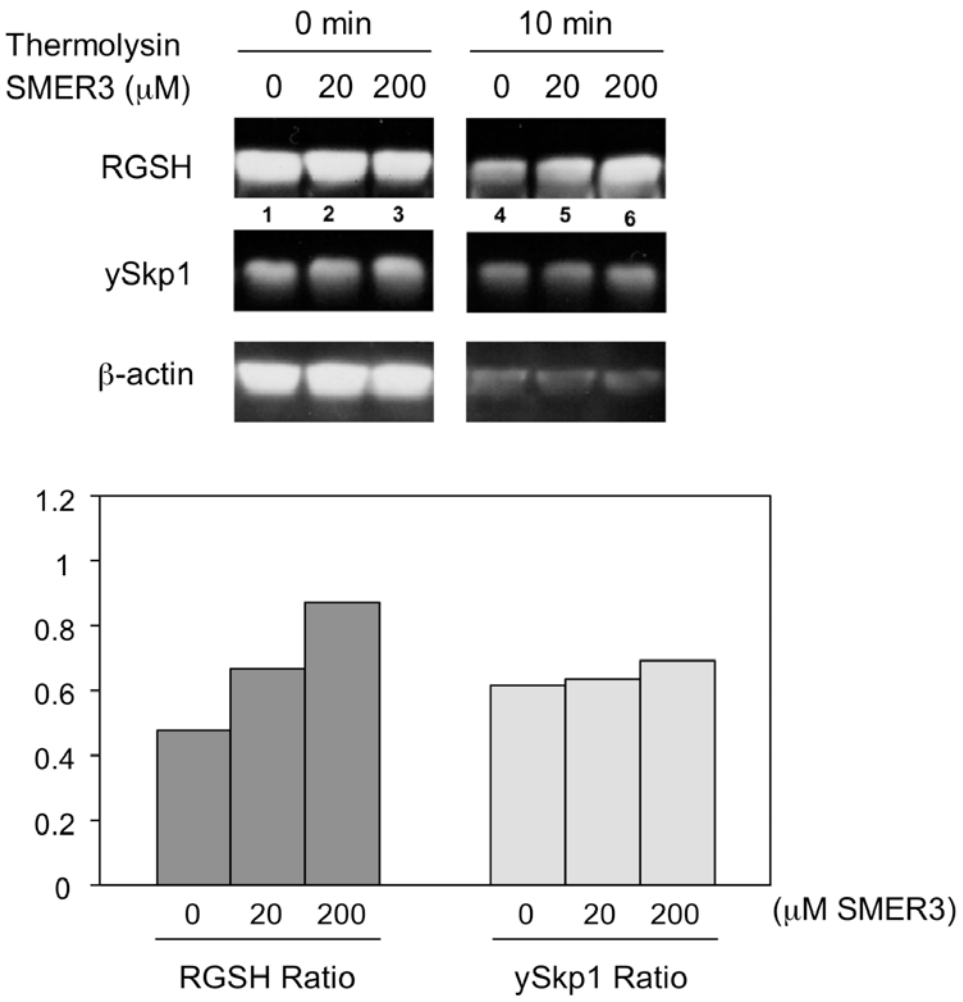

Supplementary Figure 5. Quantitative western analysis of Figure 4d showing that Met30 is protected against thermolysin digestion by SMER3. Intensities of samples that were digested for 10 min with thermolysin were normalized to those of undigested samples.

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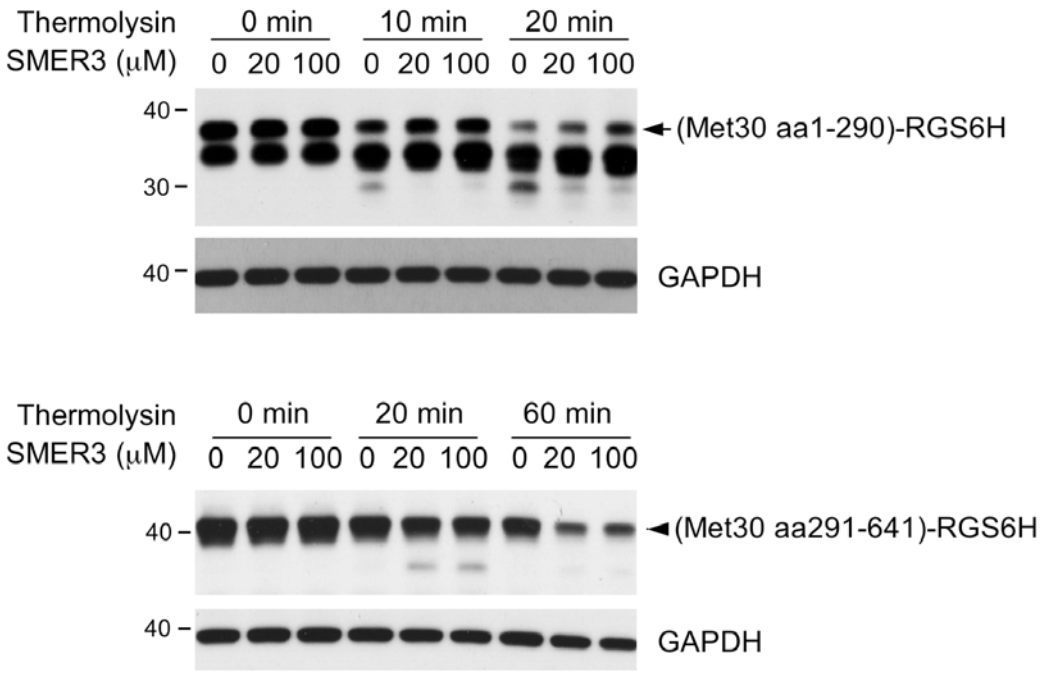

Supplementary Figure 6. SMER3 protects the F-box region, but not the WD40-repeat region, of recombinant Met30 from protease digestion. Met30 coding region 1-870 (F-box) or 871-1923 (WD-40 repeat) was PCR-subcloned into pcDNA3.1(-) (Invitrogen). Proteins were expressed using Promega TnT T7 Quick Coupled Transcription/Translation System. Thermolysin digestion was performed using translated lysate incubated with SMER3 or vehicle control, and stopped by adding EDTA pH 8.0. Samples were subjected to 4-12% NuPAGE gradient gel (Invitrogen) and Western blotted with anti-RGSH (Qiagen) and anti-GAPDH (Ambion) antibodies.

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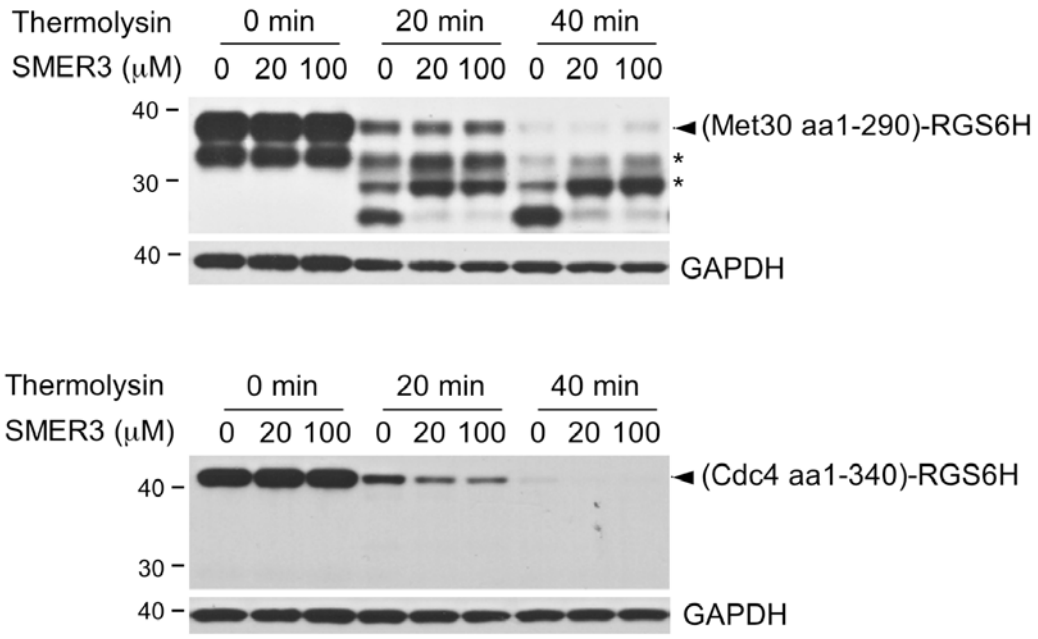

Supplementary Figure 7. SMER3 protects recombinant Met30 F-box region, but not Cdc4 F-box region, from protease digestion. Met30 coding region 1-870 (F-box) and Cdc4 coding region 1-1020 (F-box) were each PCR-subcloned into pcDNA3.1(-) (Invitrogen). Proteins were expressed using Promega TnT T7 Quick Coupled Transcription/Translation System. Thermolysin digestion was performed using translated lysate incubated with SMER3 or vehicle control, and stopped by adding EDTA pH 8.0. Samples were subjected to 4-12% NuPAGE gradient gel (Invitrogen) and Western blotted with anti-RGSH (Qiagen) and anti-GAPDH (Ambion) antibodies. The asterisks (*) indicate the Fbox(Met30) fragments that are protected by SMER3 from protease digestion.

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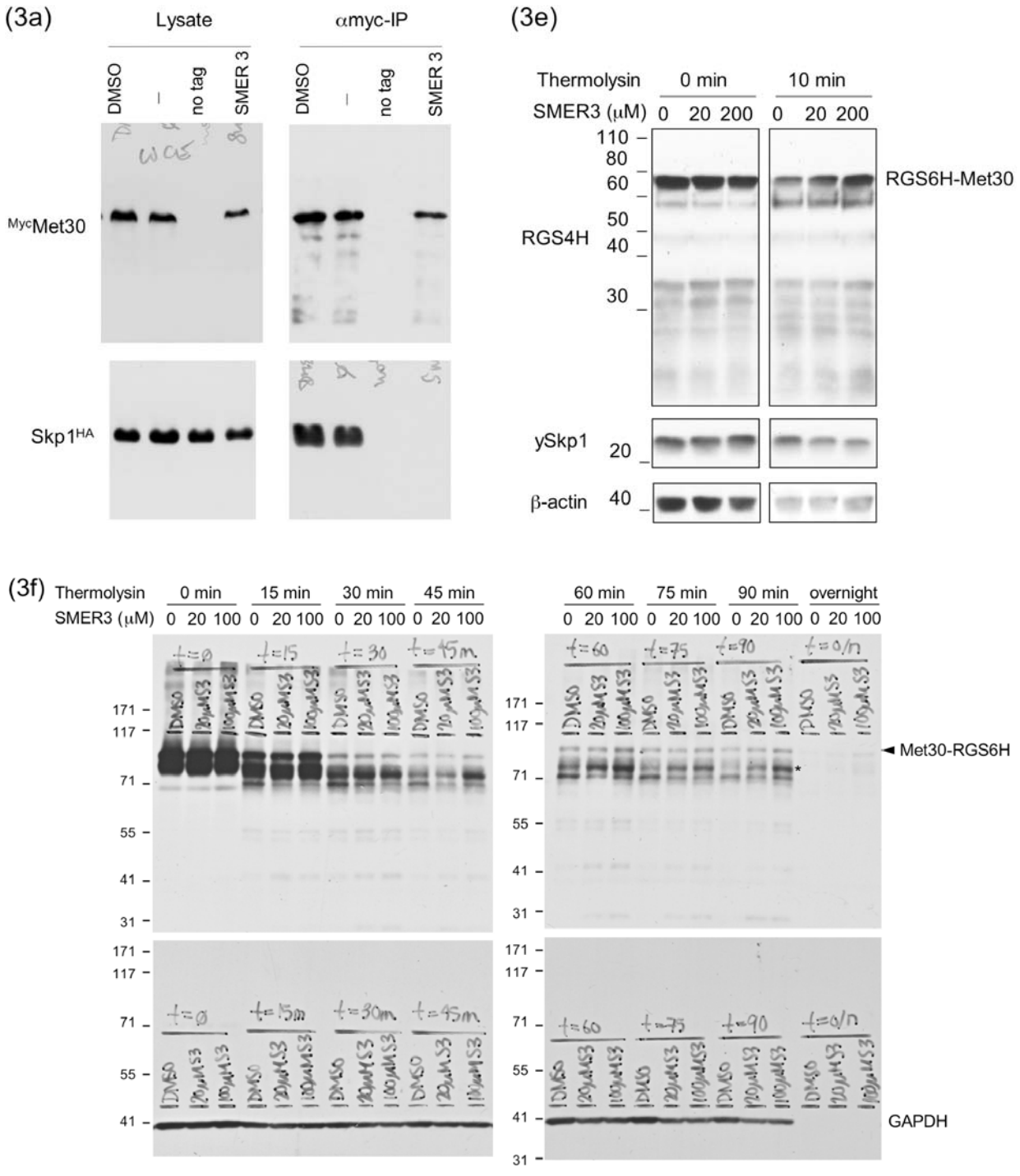
Supplementary Figure 8. Full-length blots for figure 3a,e-f.



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Supplementary Table 1. GO biological process terms enriched among the up-regulated vs. down-regulated clusters of SMER3 specific genes. p-value cutoff <0.01.

SMER3	GOID	GO_term	Expected Gene #	Observed Gene #	Cluster Gene #	GO_term Gene #	Genome Gene #	*P*-value
Up-regulated	42221	response to chemical stimulus	16	40	279	404	7159	9.96E-06
	9636	response to toxin	1	9	279	29	7159	0.00029
	42493	response to drug	5	17	279	127	7159	0.00236
	6790	sulfur metabolic process	3	12	279	71	7159	0.00496
Down-regulated	51726	regulation of cell cycle	2	9	68	158	7159	0.00269
	79	regulation of cyclin-dependent protein kinase activity	0	4	68	19	7159	0.00435

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Supplementary Table 2. Expression of *MET*-genes and cell cycle genes in cells exposed to SMER3.

Gene Name	Fold change	Description
Sulfur metabolism		
MET1	6.1	S-adenosyl-L-methionine uroporphyrinogen III transmethylase
MET2	9.5	L-homoserine-O-acetyltransferase
MET3	4.7	ATP sulfurylase
MET10	1.8	Subunit alpha of assimilatory sulfite reductase
MET14	2.7	Adenylylsulfate kinase
MET16	7.1	3'-phosphoadenylsulfate reductase
MET28	10.5	Transcriptional activator in the Cbf1p-Met4p-Met28p complex
MET32	11.6	Zinc-finger DNA-binding protein
ECM17/MET5	2.3	Sulfite reductase beta subunit
Permeases		
SUL1	4.0	High affinity sulfate permease
SUL2	3.8	High affinity sulfate permease
MUP1	1.8	High affinity methionine permease
MUP3	8.8	Low affinity methionine permease
Cell cycle		
CLN1	-2.7	G1 cyclin involved in regulation of the cell cycle
CLN3	-2.5	G1 cyclin involved in cell cycle progression
CLB1	-2.3	B-type cyclin involved in cell cycle progression
CLB2	-5.9	B-type cyclin involved in cell cycle progression
CLB3	-2.0	B-type cyclin involved in cell cycle progression
CLB5	-1.9	B-type cyclin involved in DNA replication during S phase
CLB6	-2.1	B-type cyclin involved in DNA replication during S phase
HCM1	-3.3	Forkhead transcription factor that drives S-phase specific expression
MCM10	-2.1	Essential chromatin-associated protein involved in the initiation of DNA replication
CDC6	-4.0	Essential ATP-binding protein required for DNA replication
BUR2	-2.5	Cyclin for the Sgv1p (Bur1p) protein kinase
WHI5	-2.6	Repressor of G1 transcription that binds to SBF at SCB target promoters in early G1

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